TITLE: Materials for screening of combinatorial libraries

BACKGROUND OF THE INVENTION

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In recent years, molecular imprinting has become an
increasingly attractive approach for mimicking natural
binding events [1-5]. It offers an effective means of
preparing recognition materials with binding properties
10 that resemble those of natural binding entities such as
antibodies and receptors [6-9]. By using the same types of
molecular interactions present in bio-affinity processes
(e.g., ionic interactions, hydrogen bonds, and hydrophobic
interactions), tailor-made affinity materials can be
15 designed for practically any chosen target substance.

The binding performances of molecularly imprinted polymers
(MIPs) give these materials great potential in
combinatorial approaches as recognition matrices for the
20 screening and rapid selection of ligands from a
combinatorial library [10]. The high selectivity that can be
obtained, in conjunction with the robustness, are features
that make MIPs especially suitable for this type of
application [11]. It occurred to us that these specific
25 adsorbents could be useful in the screening of chemical
combinatorial libraries (CCLs) [12], and biological
combinatorial libraries [13].

SHORT DESCRIPTION OF THE DRAWINGS

Figure 1 describes the use of a molecularly imprinted
polymer (MIP) in selective binding and screening of a

5   compound from a combinatorial library. A) The compounds of
the combinatorial library (CL1, CL2 ... CLn) are allowed to
interact with the MIP. B) One selected compound of the
library (CL1) binds more strongly to the MIP than any of
the others. C) The compounds of the library that are

10   unbound (CL2, CL3 ... CLn) by the MIP can be washed away.
D) The bound species (CL1) can be extracted.

Figure 2 shows the screening of a steroid library using a
MIP according to example 1. MIP prepared against

15   11-α-hydroxyprogesterone ($\underline{1}$). Gradient elution: 0-25 min,
dichloromethane 0.1% acetic acid (v/v); 25-30 min,
dichloromethane 0.1% - 5% acetic acid (v/v); 30-40 min,
dichloromethane 5% acetic acid (v/v), 40-45 min,
dichloromethane 5% - 0.1% acetic acid (v/v); 0.5 mL/min;

20   Sample: 20 µL, concentration: 0.8 mM of each component.
Average of two consecutive analyses. The numbering of the
species (1-12) are as follows: 11α-Hydroxyprogesterone ($\underline{1}$),
11ß-Hydroxyprogesterone ($\underline{2}$), 17α-Hydroxyprogesterone ($\underline{3}$),
Progesterone ($\underline{4}$), 4-Androsten-3,17-dione ($\underline{5}$), 1,4-

25   Androstadiene-3,17-dione ($\underline{6}$), Corticosterone ($\underline{7}$), Cortexone
($\underline{8}$), 11-Deoxycortisol ($\underline{9}$), Cortisone ($\underline{10}$), Cortisone 21-
acetate ($\underline{11}$), Cortisol 21-acetate ($\underline{12}$)

DETAILED DESCRIPTION OF THE INVENTION

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In the following, we would like to describe an invention
addressing the use of MIPs in selective binding and
screening of compounds from a combinatorial library. The

principle is outlined in figure 1, where CL1, CL2 ... CLn,
represent compounds of a combinatorial library composed of
n different compounds, and MIP represents a molecularly
imprinted polymer selective for compound CL1. In the first
5   step (step A), the compounds of the combinatorial library
is allowed to freely interact with the MIP. Under these
conditions, one of the compounds of the library (CL1) binds
more strongly to the MIP (as selected from the MIP-
preparation) than any of the others (step B). In the
10   subsequent step (step C), the remaining, not bound
compounds of the library (CL2, CL3 ... CLn) can be washed
away from the system. Finally, (step D), the compound of
the library that bound to the MIP (CL1) can be extracted.
In this way, the MIP is used as a selective screening
15   matrix for a selected compound from a combinatorial
library.

Another, non-limiting use of this methodology is the use of
MIPs for simultaneous binding of a group of molecules from
20   a library of related structures. By using several compounds
in the MIP preparation, several compounds can be
selectively bound to the MIP. Similarly, a MIP prepared
against one compound can be used to selectively bind a
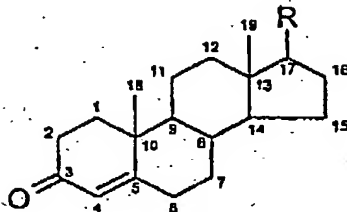group of compounds from a library.

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EXAMPLE 1

In this example, the technique was demonstrated using a
chemical combinatorial library. The combinatorial steroid
30   library used in the example is displayed in Table 1. The
library was composed of twelve closely related androsten-3-
one structures, differing only at positions 1, 11, and 17
(including sidechain). Two compounds from the library were

chosen as target molecules, 11-α-hydroxyprogesterone (1),
and corticosterone (7), and used in the preparation of MIPs
(anti-1-MIP and anti-7-MIP, respectively). Control polymers
were prepared, using the same imprinting protocol, in the
5   absence of any template steroids.

The resulting MIPs were subjected to a work-up protocol and
subsequently packed into HPLC columns. In order to verify
the order of elution, and to estimate the specificity of
10   the MIPs, individual administrations of the library species
were performed. The results from the chromatographic
evaluation of the binding specificities are displayed in
Table 2. As can be seen from these figures, it is clear
that both types of MIPs displayed high specificity with
15   respect to their respective template species. Anti-1-MIP
retained 11-α-hydroxyprogesterone longer than any other
compound in the library, and anti-7-MIP showed a similar
behavior with respect to corticosterone. As a comparison,
these compounds were not substantially retained by the
20   control polymers.

Table 1. Steroid structures

Compound	R	Other substituents
11α-Hydroxyprogesterone ($\underline{1}$)	$COCH_3$	11α-OH
11β-Hydroxyprogesterone ($\underline{2}$)	$COCH_3$	11β-OH
17α-Hydroxyprogesterone ($\underline{3}$)	$COCH_3$	17α-OH
Progesterone ($\underline{4}$)	$COCH_3$	
4-Androsten-3,17-dione ($\underline{5}$)	=O	
1,4-Androstadiene-3,17-dione ($\underline{6}$)	=O	Δ^1
Corticosterone ($\underline{7}$)	$COCH_2OH$	11β-OH
Cortexone ($\underline{8}$)	$COCH_2OH$	
11-Deoxycortisol ($\underline{9}$)	$COCH_2OH$	17α-OH
Cortisone ($\underline{10}$)	$COCH_2OH$	11=O, 17α-OH
Cortisone 21-acetate ($\underline{11}$)	$COCH_2OAc$	11=O, 17α-OH
Cortisol 21-acetate ($\underline{12}$)	$COCH_2OAc$	11β-OH, 17α-OH

Table 2. Binding specificities. Retention indices using
individual injections of 1 mM samples of the library
components. Isocratic elution: DCM 0.1% acetic acid
(anti-1-MIP), DCM 0.5% acetic acid (anti-7-MIP).

Compound	anti-1-MIP	anti-7-MIP
11α-Hydroxyprogesterone (1)	100	11
11ß-Hydroxyprogesterone (2)	13	22
17α-Hydroxyprogesterone (3)	10	8
Progesterone (4)	IS[a]	IS[a]
4-Androsten-3,17-dione (5)	-3	0
1,4-Androstadiene-3,17-dione	4	5
Corticosterone (7)	11	100
Cortexone (8)	7	41
11-Deoxycortisol (9)	8	16
Cortisone (10)	10	12
Cortisone 21-acetate (11)	7	6
Cortisol 21-acetate (12)	8	10

[a] Internal Standard

The results clearly demonstrate the efficiency of the
imprinting process. When using 11-α-hydroxyprogesterone (1)
as a print species, this compound could be easily
distinguished from the 11-ß-isomer and the 17-α-isomer.

5   Also, the anti-1-MIP could separate the print species from
corticosterone (7) and cortisone (10), which were clearly
more tightly retained by the control polymers. The results
are indicative of a high importance for the presence and
position of a hydroxyl group in the 11-position inasmuch as

10   11-ß-hydroxyprogesterone (2) showed a substantially lower
retention index than the print species did. On the other
hand, the anti-7-MIP could efficiently separate
corticosterone (7) from cortisone (10) and 11-deoxycortisol
(9), both of which were more tightly retained by the

15   control polymers. In this case, the absence of the hydroxyl
group in the 21-position (sidechain) resulted in a major
binding difference, whereas the absence of the 11-hydroxy
group resulted in considerably higher crossbinding to the
sites. Nevertheless, the recorded crossreactivities were

20   very low in all cases.

The screening capability of the MIPs was estimated upon
administration of the whole library onto the MIPs. The
results from screening the library using the anti-1-MIP are

25   displayed in Figure 2. The results clearly indicate that
the polymers were capable of selectively retaining the
template species when offered the MIPs. Thus, the anti-1-
MIP was capable of distinguishing 11-α-hydroxyprogesterone
(1) from the library, and the anti-7-MIP could selectively

30   bind corticosterone (7). In comparison, the non-imprinted
control polymers showed no significant selectivity, and
both print species were eluted well before the most tightly
retained compound (cortisone, 10). Thus, in consequence of

the molecular imprinting event, specific sites were
introduced into the polymers that were capable of
selectively fishing out the desired compounds from the
library. In spite of a close resemblance between the
5   substances, it was possible to achieve enough specificity
to distinguish small structural differences. These results
indicate that MIPs can be successfully used as synthetic
receptors in the screening of combinatorial libraries.

10   Experimental
The steroid library was purchased from Sigma (St. Louis,
MO, USA) and used as delivered. Methacrylic acid (MAA,
dried over $CaCl_2$, distilled), ethylene glycol
dimethacrylate (EDMA, dried over CaH_2, $CaCl_2$, distilled),
15   and azobis-isobutyronitrile (AIBN, used as delivered) were
from Merck (Darmstadt, Germany). Dichloromethane (DCM,
anhydrous) used in the imprinting protocol was from Lab-
Scan (Stillorgan, Ireland). All other solvents were of
HPLC-grade and used as delivered.

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Polymers were prepared using two different print molecules
(11-α-hydroxyprogesterone, 1, and corticosterone, 7), and
MAA as a functional monomer. In a typical example, the
print molecule (2.0 mmol), the functional monomer
25   (12 mmol), the crosslinker (EDMA, 60 mmol), and the
initiating agent (AIBN, 0.7 mmol) were mixed and dissolved
in the porogen (dry DCM, 18 mL). The solutions were
subsequently purged with nitrogen for 10 minutes and left
to polymerize in a Rayonet photochemical reactor (Southern
30   New England Ultraviolet Co., Bradford, CT, USA) at 350 nm
at 4 °C for 16 hours. Each polymer was ground with a
mechanical mortar (Retsch, Haan, Germany) and sieved
through a 0.025-mm sieve (Retsch). Following repeated

sedimentation in acetone, polymer particles ranging from
approximately 0.01 to 0.025 mm were collected. Control
polymers were prepared, using the same protocol, in the
absence of any print molecule.

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Each polymer was slurry-packed into a stainless steel HPLC
column (250 x 4.6 mm), and washed on-line with
methanol/acetic acid (7:3) until a stable baseline was
obtained. All analyses were performed using a Pharmacia-LKB
10 type 2249 solvent delivery system equipped with a variable
wavelength monitor model 2141 (Pharmacia-LKB Biotechnology,
Uppsala, Sweden). Chromatographic analyses were performed
either isocratically with DCM 0.1%/0.5% acetic acid (v/v),
or using gradient elution with DCM 0.1% to 5% acetic acid
15 at 0.5 mL/min at ambient temperature. Analytes were
monitored by UV absorption at 240 nm using progesterone as
an internal standard. Capacity factors (k'), and retention
indices (R.I.) were calculated using standard
chromatographic theory [14,15]. The retention index is a
20 measure of the relative retention of the analytes with
respect to both imprinted and control polymers, resulting
in a value of 100% for the template species.
R.I. = {k'$_{analyte}$(MIP)/k'$_{analyte}$(control)}/{k'$_{template}$(MIP)/k'$_{template}$(control)}.

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